them to control proton movement inside the input channels. But there are also contrasting properties; e.g. Glu286 must release a proton to render the D-channel conducting, while Lys362 takes up a proton to open the K-channel. Our comprehensive analysis of these two residues may explain several experimental observations and elucidates also general features of regulated proton conductance.

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S9.O2

The structural basis for the electron transfer function of cytochrome b561 proteins

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Cytochrome b561 (Cyt-b561) proteins are 6 transmembrane helix proteins with the four inner helices holding two b-type heme cofactors via two pairs of conserved histidine residues. The first crystal structure of a Cyt-b561 has recently been published [1]. This structure confirms general expectations about the positions of the hemes and about the ascorbate and monodehydroascorbate substrate binding sites on the opposite membrane surfaces, as derived from experimental evidence and also from an earlier modeling study [2]. We have performed homology modeling [3] based on this crystal structure of an *Arabidopsis thaliana* Cyt-b561 to obtain the structure of four other representative family members in silico: *A. thaliana* tonoplast, bovine chromaffin granule, murine duodenal and murine tumor suppressor Cyt-b561s. The extent of homology with the crystallized protein decreases in this order. A very good agreement of the backbone folding has been obtained for all four targets and the template. Transmembrane electron transfer pathways and efficiency have been calculated [4] from one bound substrate through the hemes to the other substrate. Little homology is displayed in the protein matrix along the putative electron transfer path connecting the two hemes, whereas most of the conserved amino acids are found at the heme pockets, close to the two surfaces and the substrate binding sites. The potential role of (some of the) conserved amino acids will be discussed. Acknowledgements: This work was supported by the Hungarian Scientific Research Fund (OTKA K108697).

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S9.P1

Function of the CcoP (subunit III) in the C-family O_2 reductase from *Vibrio cholerae*

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The heme–copper oxygen reductases (HCOs) catalyze the reduction of O_2, which is coupled to proton pumping across membrane and generating the proton motive force. The coordination of electron transfer and proton translocation events is crucial for the functioning of the HCOs. Many of the C-family HCOs contain a unique subunit, CcoP, that is not present in either A- or B-family HCOs. The CcoP subunit has two transmembrane spans and a hydrophilic domain that caps the periplasmic surface of the enzyme. The function of this subunit was investigated in the C-family HCO from *Vibrio cholerae*, and is shown to be important for stability as well as providing the entrance for both electrons and protons during catalysis. The hydrophilic domain of CcoP contains two heme c's that are important for electron transfer to the active site, and the hydrophobic domain contains glutamate E49, which we show is essential for proton uptake during catalysis. Whereas the 4-subunit enzyme (CcoNOQP) expresses well in a *V. cholerae* recombinant system, no expression is observed when the ccoP gene encoding the CcoP subunit is deleted. However, if only the hydrophilic periplasmic domain is deleted, leaving the transmembrane helices, the recombinant enzyme containing both the CcoN and CcoO subunits along with the hydrophobic domain of CcoP, is expressed in good yield and is stable. The truncated enzyme exhibits about 10% of the steady state oxygen reductase activity. Single-turnover flow-flash experiments with the truncated enzyme show that proton uptake is also significantly perturbed, being slower, proceeding to a lower number of protons taken up, and with a different pH-dependence. Thus, our results show that CcoP is important to stabilize the core complex of the enzyme, but also is critical to modulate the rates of the proton and electron transfer into the enzyme.

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S9.P2

Cytochrome bd oxidase from *Escherichia coli* is a quinol peroxidase mechanistically adjusted to protecting the cell from hydrogen peroxide

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Cytochrome bd oxidase is a prokaryotic terminal oxidase that catalyses the electrogenic reduction of molecular oxygen using quinol as a substrate [1]. Organisms lacking cytochrome bd oxidase show increased susceptibility to H_2O_2 and pathogenic organisms display lowered virulence. Our steady state and pre-steady state data with an ultrapure enzyme preparation show that besides its oxidase activity,